REMARKS

Claims 1-44 are pending. Claim 17 is cancelled and claims 20 and 35 are amended herein.

Objection under 37 CFR 1.75(c)

Dependent claim 35 is objected to under 37 CFR 1.75(c) as being in improper dependent form for failing to further limit the subject matter of claim 33, on which claim 35 depends. The Office Action states that claim 33 is drawn to a method of producing a protein of interest by culturing the host cells under conditions "wherein the conditions of culturing said host cell are sufficient to produce said protein of interest." The Office Action states that claim 35 "appears to be redundant in that it is drawn to 'further comprising the step of producing said protein of interest'." Applicants have amended the claim to recite "further comprising the step of *inducing the expression of* said protein of interest." The amendment is supported at page 11, lines 23-27. Applicants submit that claim 35 as amended further limits the subject matter of claim 33 affirmatively by reciting the specific step of "*inducing the expression* of said protein of interest", which leads to the production of the protein of interest as claimed. Applicants respectfully request the withdrawal of this 37 CFR 1.75(c) objection of claim 35 as amended.

Rejections under 35 U.S.C. §112, second paragraph

Claims 17 and 20 are rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite.

Claim 17 is cancelled and the phrase "Hte (high transformation efficiency) phenotype" in claim 20 is deleted. Applicants respectfully request the withdrawal of the 35 U.S.C. §112, second paragraph rejection.

Rejections under 35 U.S.C. §103(a)

Claims 1-5, 10-17, 22-23 and 26-28 are rejected under 35 U.S.C. §103(a) as being unpatentable over Del Tito et al. in view of Makoff et al. The Office Action states that the primary reference, Del Tito et al. teaches the construction and use of plasmid pRI952, which comprises an array of two tRNA genes (argU and ileX) encoding tRNAs specific for the rarely

used codons AGG/AGA and AUA, respectively. The Office Action also states that Del Tito et al. teaches that the coexpression of the two tRNA genes (ileX and argU) along with the gene encoding the heterologous polypeptide Mup^{T}IRS results in increased levels of active protein as compared to a control in which no additional tRNA genes are expressed or as compared to cells comprising a plasmid only expressing the ileX gene (Table II). The Office Action further states that Del Tito et al. teaches that "..problems in expression can be avoided by a careful inspection of the coding sequence and inclusion of appropriate tRNA genes or necessary site-specific mutations" and "that the coexpression of minor tRNAs such as ileX or argU can be utilized to overcome translational stresses due to the presence of rarely used codons within the coding sequence for a gene of interest." The Office Action states that Del Tito et al. does not explicitly teach the use of a vector comprising an array of 3 or more tRNA corresponding to rarely used codons for overexpression of a heterologous gene comprising rarely used codons, and that Del Tito et al. do not explicitly teach the use of ileX, proL, and leuW.

Makoff et al. is said to teach that the expression of the tetanus toxin fragment C in E. coli is limited by its high demand for rare tRNA molecules. The Office Action also states that Makoff et al. teaches that replacement of almost the entire coding sequence with synthetic sequence which lacks the rarely used codons results in an approximately 4 fold increase in the expression of the desired heterologous polypeptide and that fragment C from tetanus toxin shows considerable promise as a subunit vaccine against tetanus.

The Office Action concludes that it would have been obvious to one of ordinary skill in the art to modify the vector taught by Del Tito et al. with the teachings of Makoff et al. in order to express a desired heterologous polypeptide (i.e., the tetanus fragment C subunit) whose gene comprises rarely used codons by introducing additional tRNA genes corresponding to rare codons other than AGA, AGC or AUA. Makoff et al. is said to teach increasing the expression of fragment C in E. coli by compensating for the presence of a number of different rarely used codons in the gene encoding fragment C. The Office Action states that "there would have been a reasonable expectation of success in utilizing a vector made from the combined teachings above comprising 3 or more tRNA genes corresponding to the rare codons present in the coding sequence for fragment C, as taught by Makoff et al., to overexpress fragment C from its native gene in E. coli." The Office Action further states that it would have been obvious to one of skill

in the art to incorporate any tRNA gene known into the vector made from the combined teachings, and there would have been a reasonable expectation of success in utilizing such tRNA genes in the expression system made from the combined teachings to increase the expression of fragment C in E. coli. Applicants respectfully disagree.

Del Tito et al. Teaches Away from the Claimed Invention

As acknowledged by the Office Action, Del Tito et al. does not explicitly teach the use of a vector comprising an array of 3 or more tRNAs corresponding to rarely used codons in order to achieve increased expression of a heterologous gene comprising rarely used codons. In fact, Del Tito et al. do not even teach that the coexpression of two tRNA genes (ileX and argU) for rare codons results in an increase in expression of the desired heterologous polypeptides. While the authors do describe the use of a vector comprising two tRNA genes for rarely used codons, the reference actually shows that vectors comprising a single tRNA gene (either ilex or argU) for a rarely used codon results in a higher expression of a heterologous protein (flu B/LeeHA) than a vector comprising two tRNA genes.

For example, Table 1 shows that pRI952 vector containing both ileX and argU tRNAs results in much less flu B/LeeHA expression compared to other two vectors containing only ileX. The authors state that "[u]nexpectedly, the pRI952 plasmid containing both the argU and ileX genes showed a slightly diminished level of expression [of flu B/LeeHA] compared with that of the other two plasmids (i.e., ileX gene alone)...the same pattern was observed with the cultures grown in a fermentor and was a significant effect" (Page 7089, column 2, paragraph 2, lines 8-14). For the second heterologous protein tested, Table 2 of Del Tito et al. shows that pRI952 (plasmid encoding both ileX and argU genes) gives better Mup^{r}IRS expression compared to the control and compared to pI489 (plasmid encoding ileX only). However, the authors do not show Mup^{r}IRS expression in the presence of plasmid encoding argU alone. Del Tito et al. concluded from this experiment that "[t]he argU gene, which encodes an arginyl tRNA that reads AGG and AGA codons, increased specific activity more than two fold over that of pI489" (Page 7090, column 1, paragraph 2, line 12). In fact, the authors have not shown that the expression of argU tRNA alone does not result in even higher expression of Mup^{r}IRS, they do not rule out that the coexpression of argU and ilex tRNAs reduces the expression of Mup^{r}IRS, relative to its

expression with ArgU alone, which could be similar to what happened with the expression of flu B/LeeHA.

Overall, Del Tito et al. fail to show evidence that the use of two tRNA genes (i.e., ileX and argU) is more advantageous for expressing heterologous polypeptide of interest than the use of a single tRNA gene alone (i.e., either argU or ile). In fact, Del Tito et al. actually teaches away from any advantage to the coexpression of two tRNAs corresponding to rarely used codons, since the reference actually shows that the expression of a given protein is sometimes diminished when a vector comprising two tRNAs is used. Del Tito et al. thus also teaches away from a host cell containing a recombinant DNA molecule which comprises an array of three or more tRNA genes for rarely used codons, as recited in claim 1. It's improper to combine references where the references teach away from their combination, *in re Grasselli,* 713 F.2d 731, 734 (Fed. Cir. 1983).

The Combination of References Does Not Provide the Claimed Invention

Applicants submit that Makoff et al. teaches the replacement of a native gene sequence containing rare codons with a synthetic gene sequence containing more frequently used codons. Under this scheme, one may change as many codons as needed without causing any deleterious effect on the cell, provided the product is not toxic. Makoff et al. does not teach, however, a cell containing a recombinant DNA molecule which comprises an array of three or more tRNA genes, wherein said tRNA genes correspond to codons that are rarely used in said host cell. Specifically, Makoff et al. does not teach recombinant tRNA genes at all, because their approach to low frequency codon usage involves changes in the gene sequence of the gene of interest. Not only does Makoff et al. not teach recombinant tRNA genes or more than two tRNA genes, it does not teach 3 or more tRNA genes, as required by claim 1 and its dependents.

Further, Applicants submit that the combination of Makoff et al. and Del Tito et al. provides no teaching or suggestion that would lead one of skill in the art to a host cell containing a recombinant DNA molecule which comprises an array of three or more tRNA genes, as recited by claim 1. That is, Makoff et al. does not provide a teaching that overcomes Del Tito's teaching away from the claimed invention. Therefore, the combination of Del Tito et al. and Makoff et al. does not invention of claim 1 obvious.

In view of the above, neither Del Tito et al. nor Makoff et al., alone or in combination, teaches or suggests all elements of the invention claimed in claims 1-5, 10-17, 22-23 and 26-28. Applicants therefore submit that the invention as claimed in claims 1-5, 10-17, 22-23 and 26-28 is not obvious over Del Tito et al., Makoff et al., or their combination. Therefore, Applicants respectfully request that the §103 rejections of claims 1-5, 10-17, 22-23 and 26-28 over these references be withdrawn.

Claims 6-9, 19, 21 and 24-25 are rejected under 35 U.S.C. 103 (a) as allegedly being unpatentable over Del Tito et al. in view of Makoff et al. as applied to claims 1-5, 10-17, 22-23 and 26-38 above, and further in view of the 1997 Novagen catalog. The Office Action states that neither Del Tito et al. nor Makoff et al. teaches the use of a vector in which the expression of the tRNA genes is regulated by an IPTG inducible promoter, the use of a T7 RNA polymerase promoter or protease deficient cells. However, the 1997 Novagen catalog is said to describe a T7 polymerase expression system for tight control over the expression of toxic genes in E. coli. The system comprises the gene for T7 RNA polymerase under control of an IPTG-inducible promoter, a T7lac IPTG inducible promoter, and a E. coli strain lacking functional genes for Lon and OmpT proteases. The Office Action concludes that it would have been obvious to use the pET vectors/expression system described in the 1997 Novagen catalog because Del Tito et al teach that the expression of tRNA genes in E. coli can have negative effects on the host cells and because the T7 RNA polymerase-based system described in the Novagen catalog for tightly controlled expression of target, toxic genes in E. coli was well known and widely used within the art for the expression of toxic genes in E. coli.

As discussed above, none of Del Tito et al., and Makoff et al., either alone or in combination, teaches or suggests a host cell containing recombinant DNA molecule which comprises an array of three or more tRNA genes, wherein said tRNAs correspond to codons that are rarely used in said host cell as required by claim 1 and all claims dependent from it, including claims 6-9, 19, 21. Nor do they teach a vector that replicates in a host cell, said vector comprising an array of three or more tRNA genes which correspond to codons that are rarely used in said host cell as required by claim 24 and 25. Most notably, the references lack a teaching of an array of three or more tRNA genes. Therefore, unless the 1997 Novagen catalog

teaches or suggests this element, the invention of claims 6-9, 19, 21 and 24-25 cannot be obvious over any combination of these references.

Applicants submit that the 1997 Novagen catalog does not teach or suggest a recombinant molecule or a vector comprising an array of three or more tRNA genes which correspond to codons that are rarely used in host cells. The Novagen catalog describes a T7 polymerase expression system comprising the gene for T7 RNA polymerase under control of an IPTG-inducible promoter, a T7lac IPTG inducible promoter.

The 1997 Novagen catalog does not teach, however, a cell containing a recombinant DNA molecule which comprises an array of three or more tRNA genes corresponding to rarely used codons. In fact, the reference does not teach an array of tRNA genes at all. Therefore the 1997 Novagen catalog does not contribute to the element missing from the combination of Del Tito et al. and Makoff et al. and does not provide further teachings that would lead one of skills in the art to use a pET vector as a recombinant DNA which comprises an array of tRNA genes as required by claims 1 and 22, and their dependent claims. None of the references cited, either alone or in combination, teaches or suggests every element of claims 6-9, 19, 21 and 24-25. Applicants therefore submit that the invention as claimed in claims 6-9, 19, 21 and 24-25 are not obvious over any combination of these references. Applicants respectfully request withdrawal of the §103(a) rejection of claims 6-9, 19, 21 and 24-25 over these references.

Claims 18 and 20 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Del Tito et al. in view of Makoff et al. and the 1997 Novagen catalog as applied to claims 1-17, 19 and 21-38 above, and further in view of Wnendt. According to the Office Action, the references (Del Tito et al., Makoff et al., and the 1997 Novagen catalog) do not teach the use of endA$^-$ E. coli strains. However, Wnendt is said to teach the use of endA$^-$ strains for great and higher quality yields of plasmid DNAs from bacterial cells.

As stated above, none of Del Tito et al., Makoff et al. and the 1997 Novagen catalog, either alone or in combination, teaches a host cell containing a recombinant DNA molecule which comprises an array of three or more tRNA genes as required by claim 1 and its dependents. Therefore, unless Wnendt teaches or suggests the missing elements, the invention of claims 18 and 20 cannot be obvious over any combination of these references.

Applicants submit that Wnendt does not teach a recombinant DNA molecule which comprises an array of three or more tRNA genes. In fact, Wnendt does not teach the use of any array of tRNAs. Therefore, it's not obvious to one of ordinary skill in the art to make an endA deficient host cell containing a recombinant DNA which comprises an array of three or more tRNA genes from the teachings of above references, alone or in any combinations. Applicants therefore submit that the invention as claimed in claims 18 and 20 are not obvious over any combination of these references. Applicants respectfully request withdrawal of the §103(a) rejection of claims 18 and 20 over these references.

Claims 39-44 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Del Tito et al. The Office Action states that the primary reference teaches the construction and use of plasmid pRI952, which comprises an array of two tRNA genes (argU and ileX) encoding tRNA specific for the rarely used codons AGG/AGA and AUA, respectively. The Office Action further states that Del Tito et al. does not teach the use of any vector nucleic acid comprising two tRNA genes corresponding to rarely used codons other than a vector comprising argU and ileX, and that Del Tito et al does not teach the use of ileX, proL, leuW or a tRNA corresponding to rarely used glycine codons.

The Office Action concludes that it would have been obvious to one of ordinary skill in the art to modify the vector construct taught by Del Tito et al. by interchanging different tRNA genes corresponding to other rarely used codons, because Del Tito et al. teaches compensation for the presence of rarely used codons by supplying the tRNA corresponding to rarely used codons and because such rarely used codons and the genes for their corresponding tRNAs are and were known in the art. Applicants respectfully disagree.

Applicants submit that, as explained above for claims 1-5, 10-17, 22-23 and 26-38, Del Tito et al. actually teaches away from a recombinant DNA molecule or a vector which comprises an array of three or more tRNA genes by showing that the use of a vector comprising two tRNA genes encoding rarely used codons results in diminished expression of a heterologous polypeptide relative to a single tRNA vector. In addition, Del Tito et al. teaches away from the use of more than one tRNA gene encoding for rarely used codons by showing deleterious effect of these tRNA genes on the growth of host cells. Applicants submit that Del Tito et al. does not

teach the use of any vector comprising two tRNA genes corresponding to rarely used codons other than argU and ileX, and that the combination of argU and ileX is not necessarily as efficient as a single tRNA molecule, as demonstrated by Del Tito et al. Del Tito et al. does not teach the use of ileX, proL, leuW or a tRNA corresponding to rarely used glycine codons. Applicants submit that it is not obvious to one of ordinary skill in the art in view of Del Tito et al. to modify the vector construct taught by Del Tito et al. by interchanging different tRNA genes corresponding to other rarely used codons because the vector Del Tito et al used causes a deleterious effect, and the use of two tRNAs on the same vector diminishes the expression of heterologous polypeptide of interest. Therefore, Del Tito et al. and the knowledge of one skilled in the art do not make a recombinant DNA or a vector which comprises an array of three or more tRNA genes obvious. Applicants therefore submit that the invention as claimed in claims 39-44 is not obvious over Del Tito et al. Applicants respectfully request withdrawal of the §103 rejections of claims 39-44.

Commercial Sales

With respect to all of the recited 103 rejections, Applicant submits that the claimed invention has met with significant commercial success in the form of sales in the marketplace.

A showing of commercial success of a claimed invention, whenever such success occurs, is relevant in resolving the issue of nonobviousness. *Lindemann Maschinenfabrik GmbH v. American hoist & Derrick Co.*, 730 F.2d 1452, 1461, 221 U.S.P.Q. 481, 487 (Fed. Cir. 1984).

A patenee asserting commercial success of non-obviousness must demonstrate a sufficient relationship between the commercial success and the patented invention such that the success can be attributed to the invention. *Alpex Computer Corp. v. Nintendo Co. Ltd.*, 86 Civ. 1749, 34 U.S.P.Q.2d 1167, 1190 (N.Y. 1994)

A patentee need not show that all possible embodiments within the claims were successfully commercialized in order to rely on the success in the marketplace of the embodiment that was commercialized. *Applied Materials Inc. v. Advanced Semiconductor Materials*, 40 U.S.P.Q.2d 1481, 1486 (Fed. Cir. 1996).

The commercial success of a patented invention is clearly important. That evidence of such success is "secondary" in time does not mean that it is secondary in importance. Evidence of secondary considerations may often be the most probative and cogent evidence in the record. *Trustwall Systems Corp. v. Hyrdo-Air Engineering Inc.*, 813 F.2d 1207, 2 U.S.P.Q.2d 1034 (Fed. Cir. 1987).

The question is not whether evidence of commercial success is "commensurate in scope with the claims," but rather whether the evidence is relevant to the question of non-obviousness. *E.I. du Pont de Nemours & Co. v. Phillips Petroleum Co.*, 656 F. Supp. 1343, 2 U.S.P.Q.2d 1545 (Del. 1987).

Applicants have established a nexus between the claimed invention and the success. *Exparte Standish*, 10 U.S.P.Q.2d 1454 (B.P.A.I. 1988).

The evidence of commercial success consisted solely of the number of units sold. There was no evidence of market share, of growth in market share, of replacing earlier units sold by others, or of dollar amounts, and no evidence of a nexus between sales and the merits of the invention. Under such circumstances, consideration of the totality of the evidence, including that relating to commercial success, does not require a holding that the invention would have been non-obvious to one skilled in the art at the time it was made. *American Standard Inc. v. Pfizer Inc.*, 722 F. Supp. 86, 14 U.S.P.Q.2d 1673, 1715 (Del. 1989).

In the instant patent application, Applicants can not show "market share," "growth in market share," or "replacing earlier units sold" because none of these criteria existed prior to the stated sales. However, Applicants herein attest to "dollar amounts" as the nexus between product sales and the merits of the invention.

When a patented device is a commercial product, there is an inference that its commercial success is due to the patented device itself, absent a showing to the contrary. *See e.g., Hughes Tool Co. v. Dresser Indus., Inc.*, 816 F.2d 1549, 1556, 2, U.S.P.Q.2d 1396, 1402 (Fed. Cir.), cert denied, 484 U.S. 914 (1987), as interpretted in the unpublished (not citable as precedent) opinion for *Comair Rotron Inc. v. Matsushita Electric Corp. of America*, 33 U.S.P.Q.2d 1785, 1788 (Fed. Cir. 1994).

The attached 132 declaration by Ms. Mary Buchanan from the assignee of the instant invention demonstrates commercial success of the claimed invention. Since the products were launched in the marketplace in 1999, yearly total sales have been $493,441 in 1999 and $489,520 in 2000, and $41,715 to date in 2001. These significant sales evidence the nonobvious nature of the claimed methods and compositions at the time of filing.

In view of the above, Applicants submit that all patentability issues raised in the Office Action have been addressed and that the claims are in condition for allowance. Applicants respectfully request such action.

Respectfully submitted,

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